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MECHANISM OF CONTROL OF CELL DEATH AND HISTOLYSIS ASSOCIATED WITH REMODELING OF ANIMAL TISSUES

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Anura are one of unique and useful experimental animals for the study which aims at revealing the mechanism of formation of animal's body shape, because their life cycle contains the period of larva that drastically changes its body shape into adult form (metamorphosis, transformation of body morphology). At the metamorphosis, larva-specific tissues stop functioning and fall into histolysis and likewise larval cells fall into cell death (apoptosis), which occurs in a regionally and temporally regulated manner, suggesting that some specific program ("death program") governs these processes. Our study has been developed to know the entity of the death program at the cellular and molecular levels. For this aim, a special interest has been paid to the mechanism of action of thyroid hormone (TH) because the expression of death program is strictly controlled by TH.

Prolaction (PRL) has been known as an endocrine factor that stimulates the growth and development of anuran larvae. We showed that PRL is especially effective in developing connective tissues of the tadpole tail and stimulates the collagen synthesis more than 20 times. Northern blot analysis showed that PRL enhances the expression of collagen genes but suppresses that of collagenase genes. Our study supports the idea that PRL plays some role for the normal larva to adult transition by constantly activating larval cells that are engaged in "the last day job" in the regressing tail tissues.

TH triggers the death of larval cells such as tail skin cells. Therefore, it had been considered that the initial event of very complex cascades of biochemical reactions involved in the cell death is the formation of a complex of TH and thyroid hormone receptor (TR). We characterized nuclear TR of tail cells by the Scatchard analysis and demonstrated the presence of the high affinity and low capacity receptor for the first time in the amphibian. The capacity but not the affinity of TR increases at the climax stage of metamorphosis. Recently, as described below we showed the presence of cis-acting element for TR in tadpole collagenase gene.

The skin of tadpole does not show regional specificities in the morphology at the beginning of its development, but acquires the regionality during larval development: the body skin becomes different from the tail skin in their structure and metamorphic changes. We described the process of this region-specific change histologically and immunologically. Tail epidermal cells remain as larval cells and keep to express larval antigens, while the body epidermis produces epidermal pregerminative cells of adult-type and adult antigens. Larval epidermal cells and adult pre-germinative cells show quite different responses to TH: the former falls into apoptosis and the latter is stimulated to proliferate and differentiate.

The subepidermal mesenchyme was shown to induce the regional specificity of epidermis described above: the tail mesenchyme has the potency to change the back epidermis to the tail epidermis when the former is artificially combined with the latter, and *vice versa*. This mesenchymal inducing activity is lost at the early stage of premetamorphosis when TH-dependent remodeling of the mesenchyme occurs. The concentration of TH required at this stage is much lower than that required to initiate the climax change of metamorphosis.

Larva-specific tissues are subject to histolysis when plasma level of TH increases to around 10^{-9} M as of triiodothyronine. To know biochemical and molecular biological mechanism of the TH-induced histolysis, we have placed a target of the study on collagenase which is responsible for the breakdown of collagens. We purified tadpole collagenase, produced polyclonal antibodies against it and cloned cDNA and genes of the enzyme. Western and northern blot analyses showed that the expression of collagenase genes is up-regulated by TH. Immunohistology identified cells producing the enzyme as epidermal cells and mesenchymal cells in the collagen layer. The gene of anuran collagenase has a quite unique structure consisting of 4 exons as compared to the mammalian gene that contains 10 exons. Most interestingly, the tadpole gene has the thyroid hormone responsive element (TRE) in the transcription regulatory region, while the mammalian gene dose not as far as we have checked. We speculate that TH-dependent regulation of expression of collagenase is unique in amphibian. We succeeded in discovering a unique and new thiol endoprotease (ProteaseT_1) that degrades effectively and preferentially actin and characterized it biochemically. This enzyme is also TH-responsive and functions in the breakdown of muscle tissues by attacking the I band.

There still remain questions unanswered whose resolutions much contribute to the understanding of molecular and cellular mechanism of amphibian metamorphosis such as: the

origin of adult-type pre-germinative epidermal cells; the entity of mesenchymal factor which donates the regional specificity to the larval skin; transcription factors that regulate the TH-responsive and metamorphosis-associated proteins. We plan to develop studies to solve these questions.